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14. ABSTRACT

The three-dimensional structures of several types of recombinant obelin have been determined to atomic resolution. Obelin is a calcium-regulated photoprotein and the origin of the bioluminescence from the marine hydroid polyp Obelia. A W92F mutant obtained showed a violet bioluminescence emission but without change in dimensionality of the substrate binding site. The structures are typical of the super-family of calcium-binding E-F hand proteins. The high quality of the crystals also allowed a novel crystallographic method of anomalous scattering from the protein sulfur, to be demonstrated. The substrate coelenterazine is bound within the protein substituted as a 2-hydroperoxide. The NMR study indicated that the solution secondary structure did not differ substantially from the crystal.

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FINAL REPORT

GRANT #: N00014-02-1-0230

PRINCIPAL INVESTIGATOR: John Lee, Ph.D.

INSTITUTION: University of Georgia

GRANT TITLE: Crystal and Solution Structure of the Photoprotein Obelin

AWARD PERIOD: 12 March 1999 - 31 December 2002

OBJECTIVES: To solve the three-dimensional structure of the bioluminescent photoprotein Obelin. The crystal structure will be solved by X-ray crystallography provided that quality crystals can be grown, and the solution structure by high-field NMR.

APPROACH: With the collaboration of Dr. John Blinks at the Friday Harbor Labs, University of Washington, and Dr. Eugene Vysotski, Institute of Biophysics, Krasnoyarsk, Russia, obelin and a number of its mutants have been developed into high expression systems. Preliminary crystallization trials were promising and NMR results together indicated that both crystal and solution structure determinations should be feasible. Screening trials will be done to establish the conditions for obtaining the highest quality crystals. Diffraction will be measured both at-home facilities and with synchrotron beam time available at the APS and elsewhere. For the NMR investigations the proteins will be enriched with ^{13}C and ^{15}N so that the standard barrage of three-dimensional NMR experiments can be carried out for the purpose of resonance assignments. Restraints for structure will be obtained from a series of NOESY experiments. Facilities are available on campus, both 600 MHz and 800 MHz machines.

ACCOMPLISHMENTS: High quality crystals of recombinant obelin from *Obelia longissima* were produced and enabled the development of the technique of sulfur single wavelength anomalous scattering for the *de novo* analysis of protein structure in general, a significant advance in crystallographic techniques. A high-resolution (1.7 Δ) structure was determined. Crystals of a different crystal form allowed an improvement to 1.1 Δ. With access now to the APS at Argonne and application of ShelxD procedures, the structure has been now refined to 0.96 Å, the highest resolution of EF-hand proteins.

Contrary to the expectation that the bound substrate coelenterazine, should be substituted as a peroxide, the structure of obelin from *O. longissima* from two crystal forms at resolutions 1.7 Δ and 1.1 Δ, both showed electron density of only a single oxygen substituted at the 2-position of the coelenterazine. This contrasted with the

finding in the structure of the related photoprotein of a peroxy substitution, although the peroxy electron density at the 2-position was weak, but explained as due to radiation damage. Both photoproteins have overall structures typical of the calcium-regulated protein family, four helix-turn-helix motifs. Less common however, is that only three of these motifs are EF-hands with ability to bind Ca^{2+}. Cloning and expression of recombinant obelin from another species, *O. geniculata*, was carried out. The two obelins have 86% sequence identity and very similar spacial structure. A contrasting finding is the presence in the *O. geniculata* structure of strong electron density at the C2-position of the coelenterazine accounting for the two peroxidic oxygens. The two obelins differ only slightly in respect to bioluminescence sensitivity and response to Ca^{2+} with little interference by $Mg^{2+}$and low Ca^{2+}-independent luminescence. They differ in bioluminescence spectral maximum, 495 and 485 nm respectively, and in the product fluorescence, 520 and 510 nm maxima.

The W92F mutant of obelin was overexpressed, crystallized, and structure solved to 1.7 . This mutant emits a violet bioluminescence, in contrast to the blue of the wild-type obelin. A mechanism for the production of the violet bioluminescence from W92F was proposed. Mutant shows no difference in dimensionality from the wild-type, except for the absence obviously of the Trp-92 residue and again the presence of two-oxygen electron density at the C2-substitution position of the ligand. When the refinement of the wild type structure was completed to 0.96 , a weak electron density at the second oxygen became evident. The explanation for these varying densities of the peroxy oxygens is not apparent.

CONCLUSIONS: The three-dimensional structure of obelin is typical of the super-family of calcium-binding E-F hand proteins. Obelin is an unusually cooperative protein in being able to form high quality crystals for X-ray structure study, recently giving diffraction beyond 1.0 Å so that the structure could be refined to atomic resolution (0.96 Å), the highest for any E-F hand protein. The high quality of the crystals also allowed a novel crystallographic method of anomalous scattering from the protein sulfur, to be demonstrated. The substrate coelenterazine is bound within the protein substituted as a 2-hydroperoxide. The NMR study indicated that the solution structure did not differ substantially from the crystal. In spite of initial promises, technical problems frustrated the goal of solving the spatial structure by the NMR method.

SIGNIFICANCE: The spatial structures of two species of obelin, of the related photoprotein aequorin, and of an obelin mutant that emits a violet color of bioluminescence, give insight into the machinery by which calcium triggers the light emitting reaction, the method the protein uses to stabilize the intermediate hydroperoxide, and the means of

controlling the nature of the product excited state. This information provides the basis for rational mutational engineering to produce novel photoproteins with useful properties, color, temperature stability, etc.

PATENT INFORMATION: None

AWARD INFORMATION: None

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